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RNA EXTRACTION FROM WHEAT STIGMAS STORED IN DNA/RNA SHIELD

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We use this protocol and it's working

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Abstract

Despite the importance of understanding the molecular processes plants go through under field conditions, transcriptomic studies are often difficult to perform on field samples since access to liquid nitrogen is not always possible. Here, I present a protocol optimised for the extraction of high-quality RNA from wheat stigmas stored in DNA/RNA shield as a substitute to liquid nitrogen. This protocol is based on a first step of TRIzol/Chloroform phase separation and a second step to purify the aqueous phase using RNA Clean and Concentrator kit from ZYMO-Research. The addition of in-tube DNase treatment during the purification step makes this protocol also ideal for tissues with high carbohydrate content, like the wheat stigma, as DNA tends to co-precipitate with the carbohydrates and consequently overriding the on-column purification steps. This protocol has been tested in other plant tissues such as maize and wheat pollen and floral meristems in wheat with good results.

Materials

Reagents

DNA/RNA Shield™ reagent from Zymo Research
TRI Reagent® from Sigma-Aldrich
Chloroform, >= 99.8% from Fisher Scientific
RNA Clean & Concentrator-5 with DNase I Set from Zymo Research

Equipment

Refrigerated microcentrifuge (cool it at 4 °C)
RNase free tubes: microcentrifuge tubes
RNase decontamination solution like RNase AWAY® or RNaseZap®
Tissue homogenizer (such as Geno Grinder)
Steel ball bearings

Before start

RNA is not as stable as DNA and is susceptible to degradation by heat, RNases, and other enzymes.
Always wear gloves, and whenever possible, work quickly to reduce RNA degradation and work under fume hood when using TRIzol and chloroform.
Keep work area, equipment, and reagents RNase-free (RNase decontamination solution, such as RNaseZap® or RNase AWAY®, may be used).

Phase separation

1 Add two steel ball bearings into each Eppendorf tube containing the samples preserved in DNA/RNA Shield solution (~200 µL should be enough for five stigmas at Waddington stage 10 – i.e., stigmatic branches spreading outwards) (Fig. 1)

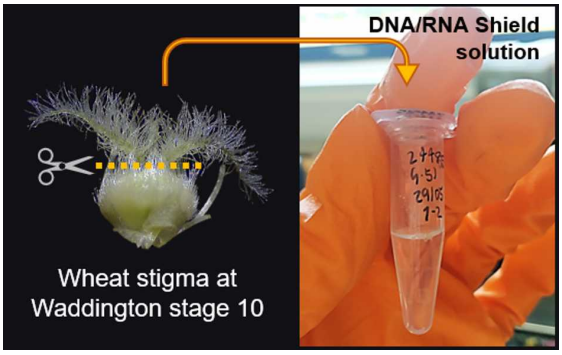

Figure 1. Diagram showing the sampling method.

2 Grind stigmas in DNA/RNA Shield solution for 2 mins at full speed in a tissue homogenizer like Geno Grinder. If foam is formed on the surface spin the Eppendorf tubes at the maximum speed for approx. 1 min in the centrifuge.

3 Add 800µL of TRIzol reagent (kept at 4 ^{O}C).

4 Mix samples by gentle inversion or rocking motion for 2-3 minutes at RT and incubate the lysate at RT for 5 min to allow complete dissociation of nucleoprotein complexes.

5 Centrifuge at 15,000 rpm (no more than 12,000x*g*) for 10 mins at 4 ^{O}C.

6 Transfer the supernatant into a fresh 1.5 mL tube. Add 0.2 volumes of chloroform per volume of TRIzol (e.g., 160 µL per 800 µL of TRIzol). Shake the tube vigorously by hand for 15 s.

7 Incubate for 2-3 min at RT.

8 Centrifugate at 15,000 rpm (no more than 12,000x*g*) for 10 mins at 4 ^{O}C.

The following steps are part of the RNA Clean and Concentrator kit (ZYMO-Research):

9 **From now onwards, perform all steps at room temperature and centrifugation at 10,000 – 16,000 x g for 30 seconds**
Following TRIzol/Chloroform extraction, carefully transfer the upper aqueous phase into a nuclease-free tube (not provided).

10 For each volume of the aqueous phase (as measured or estimated), add 1 volume ethanol (95-100%) and mix.

11 Transfer the sample to the Zymo-Spin™ IC Column in a Collection Tube and centrifuge. Discard the flow-through. To process samples >800 µl, Zymo-Spin™ columns may be reloaded.

11.1 ***In-column DNase I treatment:***
-Following the RNA binding step (step 11), prewash the column with 400 µl RNA Wash Buffer. Centrifuge and discard the flow-through.
-For each sample to be treated, prepare DNase I reaction mix in an RNase-free tube (not provided). Mix well by gentle inversion:

DNase I	5 µl
DNA Digestion Buffer	35 µl

-Add 40 µl reaction mix directly to the column matrix. Incubate at room temperature (20-30ºC) for 15 minutes. Then continue with the RNA purification protocol (step 12).

12 Add 400 µl RNA Prep Buffer to the column and centrifuge. Discard the flow-through.

13 Add 700 µl RNA Wash Buffer to the column and centrifuge. Discard the flow-through.

14 Add 400 µl RNA Wash Buffer to the column and centrifuge for 1 minute ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not provided).

15 Add 15 µl DNase/RNase-Free Water directly to the column matrix and centrifuge. Alternatively, for highly concentrated RNA use ≥6 µl elution.

16 The eluted RNA can be used immediately or stored at -80 °C to prevent degradation (avoid multiple freeze-thaw cycles).

RNA quantity and quality

17 To quantify and assess the quality of your RNA sample(s) you can use a spectrophotometer (such as a Nanodrop), agarose gel, or bioanalyzer.